February 22, 2000

VETERINARY SERVICES MEMORANDUM NO. 800.201

Subject: General Licensing Considerations: Backpassage Studies
To: Biologics Licensees, Permittees, and Applicants
Directors, Center for Veterinary Biologics

I. PURPOSE

These general licensing considerations provide guidance to applicants for conducting backpassage studies to support an application for a U.S. Veterinary Biological Product License or U.S. Veterinary Biological Product Permit for Distribution and Sale according to 9 CFR 102.5 and 104.5.

II. CANCELLATION

This memorandum cancels Veterinary Biologics General Licensing Considerations No. 800.201 dated August 29, 1995.

III. BACKGROUND

The Center for Veterinary Biologics-Licensing and Policy Development (CVB-LPD) requests that license and permit (for distribution and sale) applicants conduct backpassage studies to evaluate the genetic stability of Master Seeds for conventional modified live or live recombinant
derived vaccines to provide assurance that such vaccine microorganisms will not revert to virulence when administered to the host animal.

Backpassage studies consist of successively propagating vaccine Master Seed through a series of backpassages in vivo. Applicants administer the Master Seed microorganism to a group of host animals, and after an appropriate incubation time, recover the microorganism from these animals and administer it to a second group of host animals. Applicants should conduct a minimum of five such successive passages.

IV. GUIDELINES

A. General

1. *Study Protocols* - Applicants should submit a detailed protocol, including the criteria for determining reversion, for CVB-LPD review before initiating a backpassage study.

2. *Preliminary Data* - Applicants should submit preliminary data from studies conducted to evaluate the route of administration and procedures for recovery and to assess the expected rate of recovery of the vaccine microorganism from test animals with the proposed protocol. CVB-LPD will consider the backpassage requirement fulfilled when the applicant confirms preliminary data indicating that the applicant cannot recover the vaccine microorganism from vaccinates by using a group of 10 animals in a follow-up study performed as outlined in section IV. B. of this guideline.

3. *Passage Procedures* - In progressing from one backpassage to the next, applicants may concentrate recovered material between passages but are prohibited from in vitro propagation between passages.

4. *Study Animals* - Applicants should conduct the backpassage studies using the most susceptible species, age, and sex of animal that is in the product's label recommendations. These test animals should also be susceptible (seronegative) to the vaccine microorganism being tested.

5. *Combining Backpassage Studies with Shed-and-Spread Studies* - If the route of administration for backpassage studies determined from preliminary work is the same as the route of administration recommended on the label, applicants may expand backpassage studies to also collect data on shed and spread of the vaccine microorganism; otherwise, CVB-LPD will require a separate shed-spread study.

B. First Backpassage

1. *Route of Administration* - Administer the vaccine Master Seed to a group of host animals by the route most likely to lead to replication and to reversion of the microorganism to virulence.

2. *Numbers of Animals* - Use two to five animals, as needed, to ensure reisolation and continued backpassage (see table on probability of reisolation). Use 10 animals to confirm failure to recover the vaccine microorganism from a preliminary study (see section IV. A. 2. above).

3. *Dosage* - Administer test animals at least a typical vaccine dose (not an immunogenicity test dose).

4. *Recovery of the Microorganism* - After a time-period consistent with the pathophysiology of the progression of the disease in a naturally infected animal, attempt to recover the vaccine microorganism from the most appropriate tissues or secretions collected from treated animals.

C. Successive Backpassages

1. *Passage Procedures* - Administer recovered material (pooled material is acceptable) from animals in the preceding treatment group to animals in successive groups by the same route as in the first passage.

2. *Number of Animals for Each Successive Passage* - Based on the expected rate of recovery, treat two to five animals as needed to provide a high probability of reisolation.

3. *Observations* - Observe treated animals for clinical signs indicative of reversion of the vaccine strain to virulence.

4. *Number of Passages* - Make at least five backpassages (four successive backpassages beyond the first backpassage).

5. *Maintenance Period* - Maintain test animals from the last backpassage group for at least 21 days after administration of the recovered microorganism.

6. *Characterization* - Characterize the microorganism isolated from the last backpassage phenotypically and/or genotypically and compare it with the Master Seed to evaluate genetic stability and reversion to virulence.

/s/

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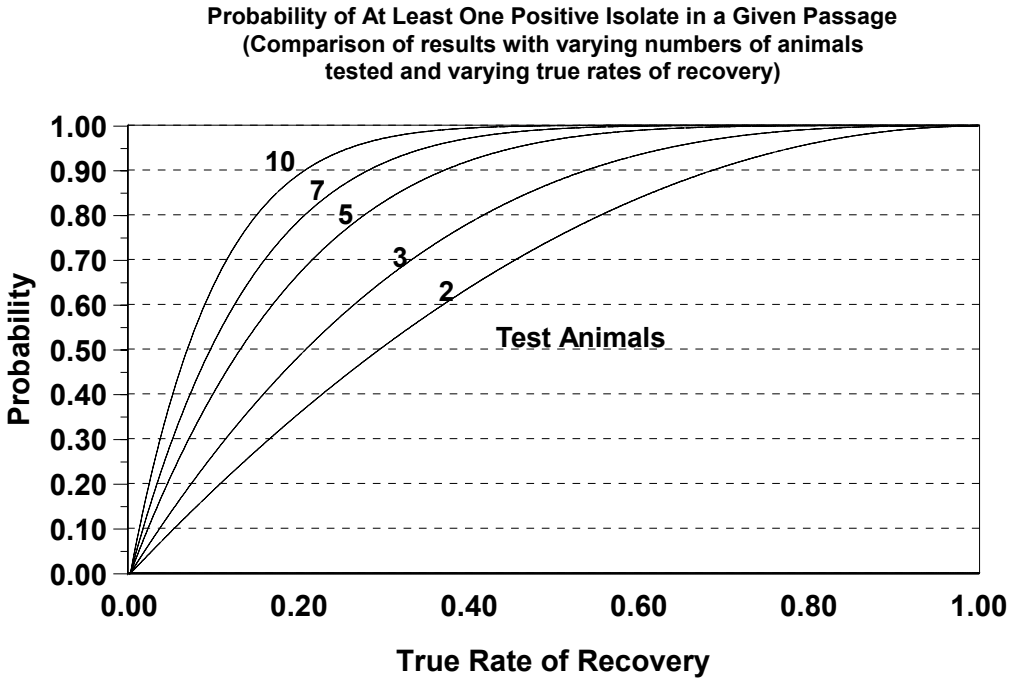
Veterinary Services

2 Enclosures

Probability of at least one positive isolate in a given passage
(Comparison of results with varying numbers of animals tested
and varying true rates of recovery)

True rate of recovery	Number of animals tested 2	3	5	7	10
0.975	0.9994	1.0000	1.0000	1.0000	1.0000
0.950	0.9975	0.9999	1.0000	1.0000	1.0000
0.925	0.9944	0.9996	1.0000	1.0000	1.0000
0.900	0.9900	0.9990	1.0000	1.0000	1.0000
0.875	0.9844	0.9980	1.0000	1.0000	1.0000
0.850	0.9775	0.9966	0.9999	1.0000	1.0000
0.825	0.9694	0.9946	0.9998	1.0000	1.0000
0.800	0.9600	0.9920	0.9997	1.0000	1.0000
0.775	0.9494	0.9886	0.9994	1.0000	1.0000
0.750	0.9375	0.9844	0.9990	0.9999	1.0000
0.725	0.9244	0.9792	0.9984	0.9999	1.0000
0.700	0.9100	0.9730	0.9976	0.9998	1.0000
0.675	0.8944	0.9657	0.9964	0.9996	1.0000
0.650	0.8775	0.9571	0.9947	0.9994	1.0000
0.625	0.8594	0.9473	0.9926	0.9990	0.9999
0.600	0.8400	0.9360	0.9898	0.9984	0.9999
0.575	0.8194	0.9232	0.9861	0.9975	0.9998
0.550	0.7975	0.9089	0.9815	0.9963	0.9997
0.525	0.7744	0.8928	0.9758	0.9945	0.9994
0.500	0.7500	0.8750	0.9688	0.9922	0.9990
0.475	0.7244	0.8553	0.9601	0.9890	0.9984
0.450	0.6975	0.8336	0.9497	0.9848	0.9975
0.425	0.6694	0.8099	0.9371	0.9792	0.9960
0.400	0.6400	0.7840	0.9222	0.9720	0.9940
0.375	0.6094	0.7559	0.9046	0.9627	0.9909
0.350	0.5775	0.7254	0.8840	0.9510	0.9865
0.325	0.5444	0.6925	0.8599	0.9362	0.9804
0.300	0.5100	0.6570	0.8319	0.9176	0.9718
0.275	0.4744	0.6189	0.7997	0.8947	0.9599
0.250	0.4375	0.5781	0.7627	0.8665	0.9437
0.225	0.3994	0.5345	0.7204	0.8321	0.9218
0.200	0.3600	0.4880	0.6723	0.7903	0.8926
0.175	0.3194	0.4385	0.6178	0.7399	0.8539
0.150	0.2775	0.3859	0.5563	0.6794	0.8031
0.125	0.2344	0.3301	0.4871	0.6073	0.7369
0.100	0.1900	0.2710	0.4095	0.5217	0.6513
0.075	0.1444	0.2085	0.3228	0.4206	0.5414
0.050	0.0975	0.1426	0.2262	0.3017	0.4013
0.025	0.0494	0.0731	0.1189	0.1624	0.2237


Probability of At Least One Positive Isolate in a Given Passage
(Comparison of results with varying numbers of animals
tested and varying true rates of recovery)
Probability
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
10
7
5
3
2
Test Animals
0.00
0.20
0.40
0.60
0.80
1.00
True Rate of Recovery